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Nucleotides in sow colostrum and milk at different stages of lactation^{1,2,3}

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ABSTRACT: An experiment was conducted with the objective of measuring the concentrations of total milk solids (TMS), CP, and 5′monophosphate nucleotides in sow colostrum and milk. Twelve multiparous sows (Landrace × Yorkshire × Duroc) were used. Litter size was standardized at 11 piglets for all sows at farrowing. Sows were fed an 18.45% CP corn–soybean meal-based diet throughout lactation. The experimental period was the initial 28 d of lactation, with colostrum collected within 12 h of farrowing and milk collected on d 3, 7, 14, 21, and 28. Colostrum and milk samples were analyzed for TMS, CP, adenosine 5′monophosphate (5′AMP), cytidine 5′monophosphate (5′CMP), guanosine 5′monophosphate (5′GMP), inosine 5′monophosphate (5′IMP), and uridine 5′monophosphate (5′UMP). Total milk solids decreased ($P < 0.05$) from 26.7% on d 0 to 23.1% on d 3. The TMS further decreased ($P < 0.05$) to 19.3% on d 7, but remained relatively constant thereafter at 18.2, 18.8, and 19.2% on d 14, 21, and 28,

respectively. The concentration of CP decreased from 16.6% in colostrum to 7.7, 6.2, 5.5, 5.7, and 6.3% in milk collected on d 3, 7, 14, 21, and 28, respectively (linear and quadratic effect; $P < 0.05$). Concentrations of 5′AMP, 5′CMP, 5′GMP, and 5′IMP increased from d 0 to d 3 and d 7, and then decreased during the remaining lactation period (quadratic effect; $P < 0.05$). The concentration of 5′UMP decreased from d 0 to 28 of lactation (linear and quadratic effects; $P < 0.05$). In colostrum, 5′UMP represented 98% of all 5′monophosphate nucleotides, and in milk, 5′UMP accounted for 86 to 90% of all nucleotides, regardless of day of lactation. The results of this experiment suggest that the concentrations of TMS and CP in sow mammary secretions changed during the first week of lactation, but were constant thereafter. Likewise, the concentrations of 5′monophosphate nucleotides changed during the initial week postpartum, but during the last 2 wk of a 4-wk lactation period, the concentrations were constant.

Key Words: Colostrum, Milk, Nucleotides, Piglets, Sow

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Introduction

The problems associated with antibiotic resistance in humans have led to a growing interest in antibiotic-free animal production worldwide. The dietary inclusion of additives that may act as alternatives to antibiotics have been suggested as ways to raise pigs without using in-feed antibiotics (Turner et al., 2001). One group of nutrients that may potentially act as an alternative to antibiotics is dietary nucleotides. The major nucleotides include adenosine 5′monophosphate (AMP), cytidine 5′mo-

nophosphate (CMP), guanosine 5′monophosphate (GMP), inosine 5′monophosphate (IMP), and uridine 5′monophosphate (UMP). Because of their role in cell division, cell growth, and modulation of the immune system, dietary nucleotides may help maintain intestinal health, and thus, reduce the incidence of enteric diseases. Indeed, dietary nucleotides were shown to decrease the prevalence of diarrhea in human infants (Carver et al., 1991; Pickering et al., 1998). It is also recognized that nucleotides are needed by an animal to respond to immunological challenges (Kulkarni et al., 1994).

Very limited information is available about the young pig's needs for nucleotides, but because of the effectiveness of dietary nucleotides in improving intestinal health and in the development of the immune system in other species, it may be speculated that nucleotides are needed by young pigs during periods of stress and immunological challenges. Nursing pigs receive dietary nucleotides via colostrum and milk, but in contrast to most other livestock species, no data on the concentration of nucleotides in sow colostrum and milk are available. Thus, the objective of the present experiment was to measure the concentration of nucleotides in the colostrum and milk of

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Table 1. Composition and calculated analysis of gestation and lactation diets (as-fed basis)

Item	Gestation diet	Lactation diet
Ingredients, %		
Corn	80.05	64.93
Soybean meal, 44%	15.75	27.50
Soybean oil	1.00	4.45
Limestone	0.90	0.90
Dicalcium phosphate	1.75	1.60
L-Lysine HCl	0.00	0.07
Salt	0.40	0.40
Vitamin premix ^a	0.05	0.05
Mineral premix ^b	0.10	0.10
Calculated nutrient composition		
ME, kcal/kg	3,354	3,523
Crude protein, %	14.12	18.45
Calcium, %	0.79	0.80
Phosphorus, %	0.66	0.67
Arginine, %	0.84	1.19
Histidine, %	0.39	0.50
Isoleucine, %	0.56	0.77
Leucine, %	1.37	1.65
Lysine, %	0.68	1.05
Methionine, %	0.24	0.29
Methionine + cysteine, %	0.51	0.62
Phenylalanine, %	0.69	0.91
Threonine, %	0.52	0.70
Tryptophan, %	0.15	0.22
Valine, %	0.67	0.88

^aVitamin premix provided the following quantities of vitamins per kilogram of the diet: niacin, 25 mg; vitamin B₁₂, 0.025 mg; pantothenic acid, 13.2 mg; riboflavin, 5.0 mg; vitamin K, 2.2 mg; biotin, 0.20 mg; vitamin A, 5,000 IU; vitamin D₃, 500 IU; and vitamin E, 44 IU.

^bTrace mineral premix provided the following quantities of minerals per kilogram of diet: Se, 0.30 mg as sodium selenite; I, 0.30 mg as potassium iodate; Cu, 25 mg as copper sulfate; Mn, 25 mg as manganese sulfate; Fe, 120 mg as iron sulfate; and Zn, 125 mg as zinc oxide.

sows and thereby establish a starting point for future studies with nucleotide supplementation to diets for young pigs.

Materials and Methods

Twelve multiparous sows (Yorkshire × Landrace × Duroc) were used in the experiment. Each sow was housed in a 1.52 × 2.13-m farrowing crate in an environmentally controlled room. A feeder and a nipple drinker were installed in front of each farrowing crate. Animal care procedures were approved by the South Dakota State University Animal Care and Use Committee (No. 02-E021). The experimental period was the initial 28 d postpartum.

Corn–soybean meal-based gestation and lactation diets (Table 1) were formulated to meet or exceed suggested requirements for all nutrients (NRC, 1998). Sows were fed 3 kg (as-fed basis) of the gestation diet once daily during the final 3 wk of gestation. The lactation diet was provided from d 110 of gestation and throughout lactation. During the initial 3 d of lactation, feed intake was restricted, but sows were allowed ad libitum access to feed thereafter. Water was available at all times.

The litter size was standardized at 11 piglets per litter within 24 h after farrowing. Mammary secretions were collected from all functional teats within 12 h of farrowing (d 0), and on d 3, 7, 14, 21, and 28 of lactation. An attempt was made to collect equal amounts of secretions from all teats. On d 0, samples were collected by hand-stripping the mammary glands when colostrum was free flowing. On the remaining collection days, 1 mL of oxytocin (20 USP units/mL, VEDCO Inc., St. Joseph, MO) was administered i.v. before milking. A total of 50 mL of fluids was collected at each collection. The collected colostrum and milk samples were divided into two subsamples and stored at –20°C.

Colostrum and milk samples were analyzed in duplicate for their concentration of Kjeldahl N (AOAC, 2000). The values for N concentration were multiplied with the protein correction factor 6.38 to calculate the concentration of CP (AOAC, 2000). The concentration of total milk solids (TMS) in colostrum and milk was determined by the Mojonnier method (Artherton and Newlander, 1977).

Nucleotide extraction was carried out in duplicate samples according to the methods of Paubert-Braquet et al. (1992). Whole milk samples were thawed and 10 mL of 0.6 N perchloric acid was added to 5 mL of milk and placed in a 30-mL centrifuge tube. The mixture was centrifuged at 17,000 × g for 10 min, the supernatant fluid was filtered through filter paper, and fat was removed using a mosquito forcep. Ten milliliters of filtrate was added to 4 mL of 1 M KOH to neutralize the acid. Following centrifugation at 17,000 × g for 10 min, the supernatant fluid was aliquoted into sample tubes and analyzed for 5′monophosphate nucleotides (i. e., AMP, CMP, GMP, IMP, and UMP) using a Waters (Milford, MA) 2690 HPLC system with a photodiode array detector. Internal standards (Sigma-Aldrich Co., St. Louis, MO) added to the defatted milk before the extraction procedures were used to correct for losses during extraction and analysis.

Data were analyzed by ANOVA using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The model included time as the main effect. Linear and quadratic effects of day of lactation on colostrum and milk concentrations of TMS, CP, and 5′nucleotides were analyzed using a contrast statement. Correlation coefficients in the PROC CORR procedure of SAS were used to identify possible correlations between the concentrations of TMS, CP, and 5′monophosphate nucleotide values. In all analyses, a *P*-value of 0.05 or less was considered significant.

Results

Results from the experiment are shown in Table 2. The concentration of TMS in the colostrum and milk from sows decreased from parturition to d 28 of lactation (linear effect; *P* < 0.001). The concentration of CP also declined from d 0 to d 3 and 7, and then reached a plateau (linear and quadratic; *P* < 0.001).

The most abundant nucleotide in sow colostrum and milk was 5′UMP. The concentration of this nucleotide decreased from 555.6 μmol/100 mL in colostrum to 104

Table 2. Concentration of total milk solids, CP ($N \times 6.38$), and 5'monophosphate nucleotides in colostrum and milk from sows at different stages of lactation^a

Item	Day of lactation						SEM	P-value	
	0	3	7	14	21	28		Linear effect	Quadratic effect
Total milk solids, %	26.7	23.4	19.4	18.2	18.8	19.2	0.71	0.001	0.142
Crude protein, %	16.6	7.8	6.2	5.5	5.7	6.3	0.41	0.001	0.001
Adenine 5'monophosphate, $\mu\text{mol}/100\text{ mL}$	4.0	11.3	12.8	6.8	4.3	3.0	2.19	0.320	0.003
Cytidine 5'monophosphate, $\mu\text{mol}/100\text{ mL}$	1.5	7.1	7.1	3.5	2.3	0.98	0.98	0.173	0.001
Guanine 5'monophosphate, $\mu\text{mol}/100\text{ mL}$	5.4	14.7	14.0	10.2	6.0	7.1	1.41	0.028	0.001
Inosine 5'monophosphate, $\mu\text{mol}/100\text{ mL}$	1.1	1.8	2.6	1.4	0.9	0.4	0.38	0.365	0.013
Uridine 5'monophosphate, $\mu\text{mol}/100\text{ mL}$	555.6	305.6	263.1	144.0	122.8	104.0	27.05	0.001	0.017

^an = 12.

$\mu\text{mol}/100\text{ mL}$ in milk on d 28 (linear and quadratic effects; $P < 0.001$ and $P < 0.05$, respectively). The concentration of 5'UMP represented approximately 98% of all 5'monophosphate nucleotides in sow colostrum and 86 to 90% in milk. Compared with 5'UMP, much lower concentrations of 5'AMP, 5'CMP, 5'GMP, and 5'IMP were found. The concentrations of these nucleotides increased from d 0 to d 3 and 7 and decreased between d 7 and 14, but thereafter, the concentration remained constant (quadratic effect; $P < 0.05$).

There is a strong correlation ($P < 0.05$) between the concentration of TMS and CP in sows' colostrum and milk (data not shown). However, no significant correlations between the concentration of CP and the analyzed 5'monophosphate nucleotides were detected.

Discussion

The concentration of TMS and CP found in colostrum and milk in the current experiment are in agreement with the findings of other researchers (Bowland et al., 1949; Klobasa et al., 1987). This indicates that TMS and CP of colostrum and milk in swine have remained relatively constant over the last 50 yr, despite changes in genetics, housing systems, productivity, and feed formulations. Therefore, the nucleotide concentrations found in the current study are believed to be representative for colostrum and milk from swine in general.

The concentrations of 5'AMP and 5'CMP in sow colostrum and milk were in the range of those seen in other species (Johke, 1963; Gil and Sanchez-Medina, 1981). However, 5'GMP and 5'UMP concentrations in porcine colostrum and milk observed in this study were higher than in other species (Johke, 1963). The concentration of 5'IMP has not been reported in other species. Inosine 5'monophosphate can be synthesized from AMP and can be further transformed into other cellular metabolites, such as inosine through nucleotidases or alkaline phosphatase enzymes (Thorell et al., 1996). The detection of 5'IMP in this study indicates either that the conversion of AMP to IMP via the enzyme AMP deaminase may be more efficient in sows than in other species or that the IMP-degradation enzymes are inefficient in swine.

The concentration of 5'UMP in sow colostrum and milk observed in this experiment is higher than in other species (Johke, 1963; Atwood et al., 1991). This suggests that species-specific secretory mechanisms may be present. Catabolic enzymes, such as kinases for specific nucleotides, may have been present during the process of secretion, accumulation, and storage within the mammary gland and may have contributed to the nucleotide concentration as well. This hypothesis was suggested by Thorell et al. (1996), who identified the presence of catabolic enzymes in milk responsible for the conversion of purine nucleotides to uric acid.

The changes throughout lactation in the concentration of 5'UMP were similar to the changes in TMS. This may indicate that the concentration of 5'UMP in sow milk is associated with lactose synthesis. During the synthesis of lactose and sialyllactose, which occurs in the Golgi vesicles, 5'UMP and 5'CMP are formed and may be released into the alveolar lumen during exocytosis (Arthur et al., 1991). Lactose and uridine diphosphate (UDP) are formed in the Golgi apparatus of the cell from glucose and UDP galactose. Uridine diphosphate is eventually broken down into UMP and inorganic phosphate. Therefore, an increased production of lactose produces an increase in UDP substrates that are enzymatically degraded to UMP. As a consequence, the concentration of 5'UMP is expected to be correlated to lactose production, and thus, to TMS. The results from the current experiment support this hypothesis.

The changes in the concentration of 5'AMP, 5'CMP, 5'GMP, and 5'IMP during lactation differed from those observed for CP, TMS, and 5'UMP. This observation suggests that the concentration of these nucleotides is not related to the production of milk lactose or protein.

Nucleotides in milk may originate from two different sources (i.e., dietary sources or nucleotides synthesized de novo). The decreased concentration of nucleotides in milk during the latter stages of lactation may reflect decreased de novo synthesis because the diet was not changed and the feed intake of the sows was constant during this period (data not shown). This decrease in de novo synthesis may be a response to a reduced need for nucleotides by the nursing piglets. However, the decrease

in milk nucleotide concentration could also be a response to an increased utilization of nucleotides within the mammary gland, where they are used as a substrate for the synthesis of DNA (Voet and Voet, 1995). Because the concentration of DNA within the mammary gland increases from d 5 to 21 of lactation (Kim et al., 1999), more nucleotides are needed for DNA synthesis during this period, which might be the reason why the concentration of nucleotides in milk decreased.

From the above discussion, it follows that it is uncertain whether the nucleotides present in sow's milk are secreted in response to a specific need of the piglets or whether they are secreted into the milk because the mammary gland has no other use for them. Nevertheless, the data from this experiment indicate the amounts of nucleotides that nursing piglets receive before weaning. Therefore, these data can serve as a starting point for future studies aimed at investigating if intestinal health and pig performance can be improved by the inclusion of dietary nucleotides in diets for pigs during the post-weaning period.

Implications

The relatively constant concentration of nucleotides in the milk of sows during the second half of lactation indicates that the supply of nucleotides to nursing piglets is relatively constant. Therefore, the 5' monophosphate nucleotide concentration found in this study may serve as a starting point for nucleotide supplementation studies in weanling pigs. It has also been established that crude protein is not a good indicator of the nucleotide concentration in sow colostrum and milk.

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